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Where Does Innate Immunity Stop and Adaptive Immunity Begin?

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The regulation of alternative splicing in the immune effector Dscam reported by Dong et al. (2012) in this issue of *Cell Host & Microbe* raises important questions about the nature of immune responses. Can we clearly define "adaptive" as being different from "innate" immunity, or is it time for a more flexible description?

Many papers on invertebrate immunity contain a statement that invertebrates are ideal for studying innate immunity because they lack adaptive immune responses. In actuality, invertebrates have spectacularly plastic immune effectors that can generate true novelty and functional immune response changes in relation to past experience. The paper by Dong et al. (2012) in this issue of *Cell Host & Microbe* concerning the mechanism behind alternative splicing of an immune effector, Dscam, adds a mechanistic dimension to the observed plasticity of invertebrate immunity. However, perhaps the most important contribution of the Dong et al. (2012) study is the demonstration of how difficult it is to divide immune responses into strictly "innate" and "adaptive" properties.

Why might authors write that flies, worms, snails, or sea urchins lack an adaptive immune response? Typically, the argument takes the following form: B and T cells generate our adaptive immune responses, therefore organisms with B and T cells likely also have an adaptive immune response. That is good logic. Organisms lacking B and T cells are often said to lack adaptive immunity, but it is a logical fallacy to conclude that just because an organism lacks these cells, the organism will also lack an adaptive immune response. These organisms could simply generate a trained immune response in another way. And they do.

To describe immunity, we plotted known immune responses according to their properties on two axes: molecular specificity and memory. A historical method of dividing innate from adaptive immunity is to discuss the specificity of the immune effectors. One could say that innate immune effectors are germline encoded while adaptive immune effectors are not. This implies that an innate system has a limited capacity to generate specific effectors whereas the adaptive immune system can create bespoke effectors over the course of the host's life span. This approach doesn't create two simple categories; there is a continuum of possibilities and no clear boundary that can be used to draw a border between adaptive and innate (Figure 1). We consider five overlapping categories along this continuum: first, there are single immune effectors, like lysozyme or reactive oxygen, which work nonspecifically against a class of microbes. Second, there are clouds of immune effectors comprised of many effectors, like antimicrobial peptides or natural antibodies. Third, there are effectors like antibodies or VLRs that can be produced via recombination or gene conversion. Fourth, antibodies and snail FREPS can be altered through somatic mutation to further increase the range of specificity (Zhang et al., 2004). Finally, there is RNAi, which produces highly specific antiviral effectors de novo. This is arguably the highest form of specificity found in an immune system because it produces tailor-made effectors from the pathogen and has no template in the host. This is as far from innate as a host can get.

The memory axis can be divided into four categories that are also drawn as a continuum in the plot in Figure 1. First, immune effectors can be expressed constitutively. Second, effectors can be stereotypically induced upon exposure to microbes. Third, effectors can persist following an immune response. This provides a sort of switch where the challenged animal is different from a naive animal; for example, amplification of specific NK cells directed toward fighting cytomegalovirus (CMV) fits this sort of model (Sun et al., 2009). Finally, there are responses that are trained by past experience and increase upon a second exposure. Antibody production following a secondary infection is an obvious example of this sort of training. Neuroimmune reflexes could be placed provocatively at this end of the spectrum as they open the possibility of neural memory affecting the immune response (Tracey, 2009). There is already evidence for this in *C. elegans* (Zhang et al., 2005).

All of this is relevant to the Dscam gene in insects because Dscam fits poorly into our classical descriptions of immunity. Considerable work has been published on the role of Dscam molecules in wiring the *Drosophila* nervous system, but Dscam serves a second role as an immune effector, and this has been less well studied (Dong et al., 2006; Watson et al., 2005). The Dscam gene in invertebrates is a complex gene that undergoes alternative splicing. Theoretically, the Dscam gene can generate up to 38,016 splice variants in dipterans (Dong et al., 2006; Watson et al., 2005). Dscam can be secreted where it can potentially act as an opsonin, and in a membrane-bound form it can potentially act as a phagocytic receptor. Dscam is reported to be functionally important in several invertebrate systems including *Drosophila* and the mosquito. In the absence of Dscam, these hosts (or at least their cells) have trouble raising an effective immune response (Dong et al., 2006; Watson et al., 2005).

Ever since Dscam was implicated in invertebrate immunity, there has been some question about how much specificity the insect can squeeze out of these molecules. Do these act like antibodies,

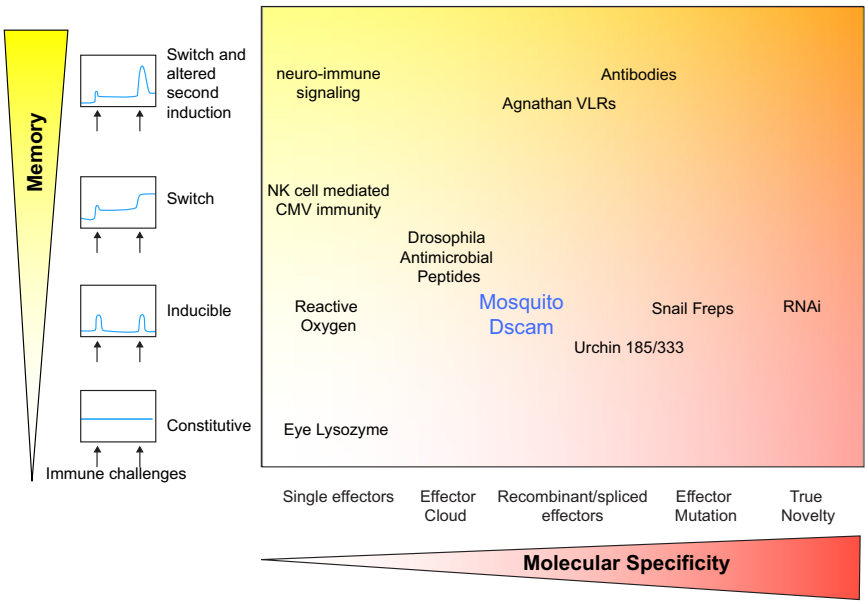

Figure 1. A Map of Immune Effectors in Memory by Specificity Space
Immune responses are plotted qualitatively against two axes, memory and specificity. The memory axis imagines induction profiles for two successive immune responses and categorizes the responses with regard to how the host responds the second time it encounters a pathogen. The specificity axis reports how many specific molecules can be produced as immune effectors and whether they are germline encoded, partially encoded, or generated de novo upon an immune response. A discussion of snail FREPS and sea urchin 185/333 molecules can be found in Buckley et al. (2008) and Zhang et al. (2004). See text for more details.

where the most specific antibody is expressed in response to stimulation by an antigen? Or is this just potential specificity that is never realized because Dscam is just expressed in the same cloud upon every exposure? Dong et al. demonstrate that the answer lies in between these two extremes.

The authors of Dong et al. (2012) find that different immune elicitors generate different splicing patterns of Dscam, tracing this through the pattern recognition pathways to the splicing factors involved. Induction through the insect immune deficiency (IMD) pathway regulates splicing of Dscam through the splicing factors Caper and/or IRSF1. The authors demonstrated that Dscam alternative splicing is important for protection of the host from infection. If the host is unable to splice appropriately for a given microbe, then the host is more susceptible. The authors present a model whereby the nature of pattern recognition signaling leads to the expression of different clouds of alternatively spliced Dscam molecules. Therefore, Dscam defies simple descriptions. Dscam is a non-germline-encoded immune effector and resembles antibodies in this respect; however, Dscam is not expressed as a single highly specific molecule, but rather it is produced as a cloud of effectors with different specificities.

The paper by Dong and colleagues places Dscam near the middle of Figure 1. The memory axis was not studied in this paper; the authors report only what happens during a primary immune response. It would be interesting to know how Dscam affects a second immune response. Does the mosquito do better upon a second challenge of the same pathogen because there is existing Dscam protein from the previous challenge? Is the correct cloud of Dscam expressed more intensely upon re-exposure? Does the mosquito do worse upon a challenge with a second different pathogen because the splicing is now directed toward another pathogen?

The best-studied effectors in Figure 1 cover large swaths of the specificity by memory space. For example, *Drosophila* antimicrobial peptides (AMPs) are expressed as a cloud at the constitutive, inducible, and switch levels. Antibodies, considered as a class of molecules, work across the whole memory axis, from constitutive expression to altered second induction. We need to fill in the missing spots for Dscam and all other immune effectors.

There is a third axis that we left off Figure 1: function. Some of the effectors placed on the plot, like snail FREPS or sea urchin 333/118 molecules, have interesting molecular specificity properties, but these have not been shown to functionally affect memory. In contrast, there many examples of functionally more effective immune responses in invertebrates where we do not have enough mechanistic information to place them on the memory and specificity axes (Pham et al., 2007; Rodrigues et al., 2010). This figure will become more complete as we resolve how each of these systems work.

Studies such as the one from Dong et al. (2012) should provoke immunologists to reassess their definition of "adaptation" and to look for signs of trained immunity (Netea et al., 2011) in systems that were originally considered innate and stereotypical.

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